UNCLASSIFIED

SEC[illegible] OF THIS PAGE

IMENTATION PAGE DTIC FILE COPY

Form Approved
OMB No. 0704-0188

1a. AD-A189 357

1b. RESTRICTIVE MARKINGS

2a. SECURITY [illegible]

3. DISTRIBUTION/AVAILABILITY OF REPORT
Distribution unlimited - approved for public release

2b. DECLASSIFICATION/DOWNGRADING SCHEDULE

4. PERFORMING ORGANIZATION REPORT NUMBER(S)

5. MONITORING ORGANIZATION REPORT NUMBER(S)

6a. NAME OF PERFORMING ORGANIZATION
U.S. Army Medical Research Institute of Infectious Diseases

6b. OFFICE SYMBOL (If applicable)
SGRD-UIS

7a. NAME OF MONITORING ORGANIZATION
U.S. Army Medical Research and Development Command

6c. ADDRESS (City, State, and ZIP Code)
Fort Detrick, Frederick, MD 21701-5011

7b. ADDRESS (City, State, and ZIP Code)
Fort Detrick, Frederick, MD 21701-5012

8a. NAME OF FUNDING/SPONSORING ORGANIZATION

8b. OFFICE SYMBOL (If applicable)

9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER

8c. ADDRESS (City, State, and ZIP Code)

10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.

11. TITLE (Include Security Classification)
Stability of T-2, HT-2, and T-2 Tetraol in Biological Fluids

12. PERSONAL AUTHOR(S)
Judith G. Pace and Charles F. Matson

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
progress	FROM _____ TO _____	1 April 1987	12

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The stabilities of tritium-labeled T-2, HT-2, and T-2 tetraol were studied in blood and urine at -70°, 4°, and 23°C for 6 months in the presence of EDTA or NaF. Samples were counted with a radiochromatographic scanner with results indicating the stability of T-2 tetraol > T-2 > HT-2. Toxins were most stable when stored at -70°C, in the presence of NaF, in urine (pH 6), then saline (control pH 7), and blood (pH 8). These results suggest that urine and T-2 tetraol are the biological fluid and metabolite of choice for diagnostic purposes.

DTIC ELECTE JAN 20 1988 S D E

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT
[ ] UNCLASSIFIED/UNLIMITED [X] SAME AS RPT [ ] DTIC USERS

21. ABSTRACT SECURITY CLASSIFICATION
Unclassified

22a. NAME OF RESPONSIBLE INDIVIDUAL

22b. TELEPHONE (Include Area Code)

22c. OFFICE SYMBOL

DD Form 1473, JUN 86 Previous editions are obsolete. SECURITY CLASSIFICATION OF THIS PAGE

UNCLASSIFIED

Stability of T-2, HT-2, and T-2 tetraol in Biological Fluids

JUDITH G. PACE* AND CHARLES F. MATSON

U.S. Army Medical Research Institute of Infectious Diseases

Fort Detrick, Frederick, Maryland 21701-5011

Running Title: T-2 stability in biological fluids

* Corresponding author: (301) 663-7181

The stabilities of tritium-labeled T-2, HT-2, and T-2 tetraol were studied in blood and urine at -70°, 4°, and 23°C for 6 months in the presence of EDTA or NaF. Samples were counted with a radiochromatographic scanner with results indicating the stability of T-2 tetraol > T-2 > HT-2. Toxins were most stable when stored at -70°C, in the presence of NaF, in urine (pH 6), then saline (control, pH 7), and blood (pH 8). These results suggest that urine and T-2 tetraol are the biological fluid and metabolite of choice for diagnostic purposes.

Recently, there has been an increased awareness of possible human exposure to trichothecene mycotoxins (10). These 12,13-epoxides are skin penetrants (2, 9) and pose a danger to crop handlers and livestock (2,3,4). Outbreaks, such as alimentary toxic aleukia (ATA) (7,13) and other incidents of human and animal exposure (3,4,10), required medical researchers to develop methods for analyzing biological fluids for the presence of trichothecene toxins. Under ideal conditions samples are treated with an anti-coagulant (EDTA or NaF) and/or antibacterial agent (thymol and F-) and stored refrigerated or frozen. However, under adverse field conditions these criteria are difficult to meet, and specimens may take weeks to reach the analytical laboratory (10). The stability of these toxins in biological fluids is not known. Therefore, by using radiolabeled trichothecene mycotoxins (8,11), we examined the stability and degradation products of T-2, HT-2, and T-2 tetraol in urine and blood stored over 6 months at -70°, 4°, and 23°C, with and without the addition of NaF. These studies were done to define the sampling and storage requirements of biological fluids used in diagnosis of trichothecene mycotoxin exposure.

Radiolabeled HT-2 and T-2 tetraol were prepared by alkaline hydrolysis of [^{3}H]T-2 (Amersham Corp., Arlington Heights, Ill.) (spec. act. 8.9 Ci/mmole of α-T-2, radiochemical purity 95%) (8,11). The purity of radiolabeled toxins was determined by high-pressure liquid chromatography (HPLC) and gas-chromatography/mass spectrometry (GC/MS) to be 95% [^{3}H]HT-2, and 99% [^{3}H]T-2 tetraol. The purified toxins were dissolved in methanol and used immediately.

Blood was collected from a 43-year-old, healthy male volunteer by venipuncture in sterile, evacuated tubes containing either EDTA or NaF. Urine (50 ml) from the same volunteer was collected in a sterile specimen cup containing EDTA or 1.25 g of NaF. Radiolabeled T-2, HT-2 or T-2 tetraol [illegible]

added to a 15-ml sample of blood, urine, or saline. Each sample was divided into three aliquots and stored at room temperature (23°), 4°, or -70°C. The frozen samples were divided into eight 0.5 ml aliquots to avoid any freeze-thaw effects. At 0, 1, 3, 7, 14, 28, 60, and 180 days, a 0.5-ml sample was removed, treated with 0.05 ml of 6% trichloroacetic acid (TCA), and centrifuged (5,000 x g, 10 min.) to remove protein. The supernatant (10 µl) was spotted on thin layer chromatography (TLC) plates, developed, and scanned for radioactivity using a Bioscan BID 200 radioisotopic scanner (Washington, D.C.) (5).

Percent of total radiolabeled T-2, HT-2, and T-2 tetraol (TOL) recovered in urine, blood, and saline stored for 28 and 180 days at 23°, 4°, and -70°C in the presence of NaF is shown in Figure 1. Refrigeration (4°C) and freezing (-70°C) prolonged the life of radiolabeled toxins. In the presence of NaF, greater than 93% of the parent toxins added to urine (pH 5) were recovered at all temperatures for up to 180 days (Fig. 1, top). There was no difference in recovery of parent toxin in the presence of EDTA (data not shown). Blood (pH 8) was metabolically active in degrading T-2 and HT-2 at 23° and 4°C in the presence of NaF (Fig. 1, middle). T-2 tetraol was stable in blood under all conditions studied for up to 6 months (Fig. 1, black bar).

Previous studies (6, 12) determined the stability of trichothecenes prepared in tissue culture media and methanol. Our results show degradation products are also found if trichothecenes are diluted and stored in saline. Toxins stored in a neutral aqueous control, saline (pH 7), were less stable at 23° than at 4° and -70°C (Fig. 1, bottom). In the presence of NaF, 84% of T-2, 80% of HT-2, and 97% of T-2 tetraol remained after 6 months storage at -70°C. In the presence of EDTA, 93% of T-2, 85% of HT-2, and 80% of T-2 tetraol remained (data not shown). The major degradation products of T-2 and HT-2 are HT-2 and T-2 triol, respectively.

Trusal (6) has shown that addition of serum to aqueous tissue culture medium increases T-2 toxin breakdown at physiological temperatures. Compared to EDTA, NaF increased the stability of T-2 in blood (Fig. 2, B and D), but had no effect on the long-term storage of HT-2 (data not shown). In the presence of EDTA at 4°C, T-2 toxin degraded to HT-2 and T-2 tetraol, while in the presence of NaF, HT-2 was the only detectable degradation product (Fig. 2).

The present study defines how biological samples should be collected and stored for the most accurate analysis of T-2 toxin and its metabolites. T-2 toxin was stable in blood and urine if stored in the presence of NaF at -70°C (Fig. 1). The degradation products most likely to be detected in blood samples collected and stored under adverse conditions were HT-2 and T-2 tetraol. In vivo, T-2 mycotoxin is rapidly metabolized ($t_{1/2}$ = 6 min) to more polar metabolites (5). T-2 tetraol is a major metabolite found in excreta of animals after exposure to T-2 toxin by parenteral or dermal routes (1, 5, 14, and Pace et al., The Toxicologist 6:246, 1986). Our data showed T-2 tetraol to be the most stable metabolite (Fig. 1, black bar) and, therefore, the most appropriate metabolite for diagnostic testing.

LITERATURE CITED

1. Corley, R. A., S. P. Swanson, and W. B. Buck. 1985. Glucuronide conjugates of T-2 toxin and metabolites in swine bile and urine. J. Agric. Food Chem. 33:1085-1089.

2. Kemppainen, B. W., R. T. Riley, and J. G. Pace. 1984. Penetration of [^{3}H] T-2 toxin through excised human and guinea pig skin during exposure to [^{3}H]T-2 toxin adsorbed to corn dust. Food Chem. Toxicol. 22:893-896.

3. Mortimer, P. H., J. Campbell, M. E. Di Menno, and E. P. White. 1971. Experimental myrothecrotoxicosis and poisoning in ruminants by verrucarin A and roridan A. Res. Vet. Sci. 12:508-515.

4. Obegonic, L., R. Pavlovic, and B. Miloseu. 1971. Toxic dermatitis, conjunctivitis, rhinitis, pharyngitis, and laryngitis in fattening cattle and farm workers caused by mold from contaminated straw (stachybotryotoxicosis). Veterinaria (Sarajevo), 20:263-267.

5. Pace, J. G., M. R. Watts, E. P. Burrows, R. E. Dinterman, C. Matson, E. C. Hauer, and R. W. Wannemacher, Jr. 1985. Fate and distribution of [^{3}H]-labeled T-2 mycotoxin in guinea pigs. Toxicol. Appl. Pharmacol. 80:377-385.

6. Trusal, L. R. 1985. Stability of T-2 mycotoxin in aqueous media. Appl. Environ. Microbiol. 50:1311-1312.

7. **Ueno, Y.** 1977. Trichothecenes: overview address, p. 189-207. In J. V. Rodricks, C. W. Hesseltine, and M. A. Mehlman (ed.), Mycotoxins in human and animal health. Pathotox, Park Forest South, Illinois.

8. **Wallace, E. M., S. V. Pathre, C. J. Mirocha, T. S. Robinson, and S. W. Fenton.** 1977. Synthesis of radiolabeled T-2 toxin. J. Agric. Food Chem. 25:836-838.

9. **Wannemacher, R. W., Jr., D. L. Bunner, J. G. Pace, H. A. Neufeld, L. H. Brennecke, and R. E. Dinterman.** 1985. Dermal toxicity of T-2 toxin in guinea pigs, rats, and cynomolgus monkeys, p. 423-432. In J. Lacey (ed.), Trichothecenes and other mycotoxins. J. Wiley and Sons, Ltd., New York.

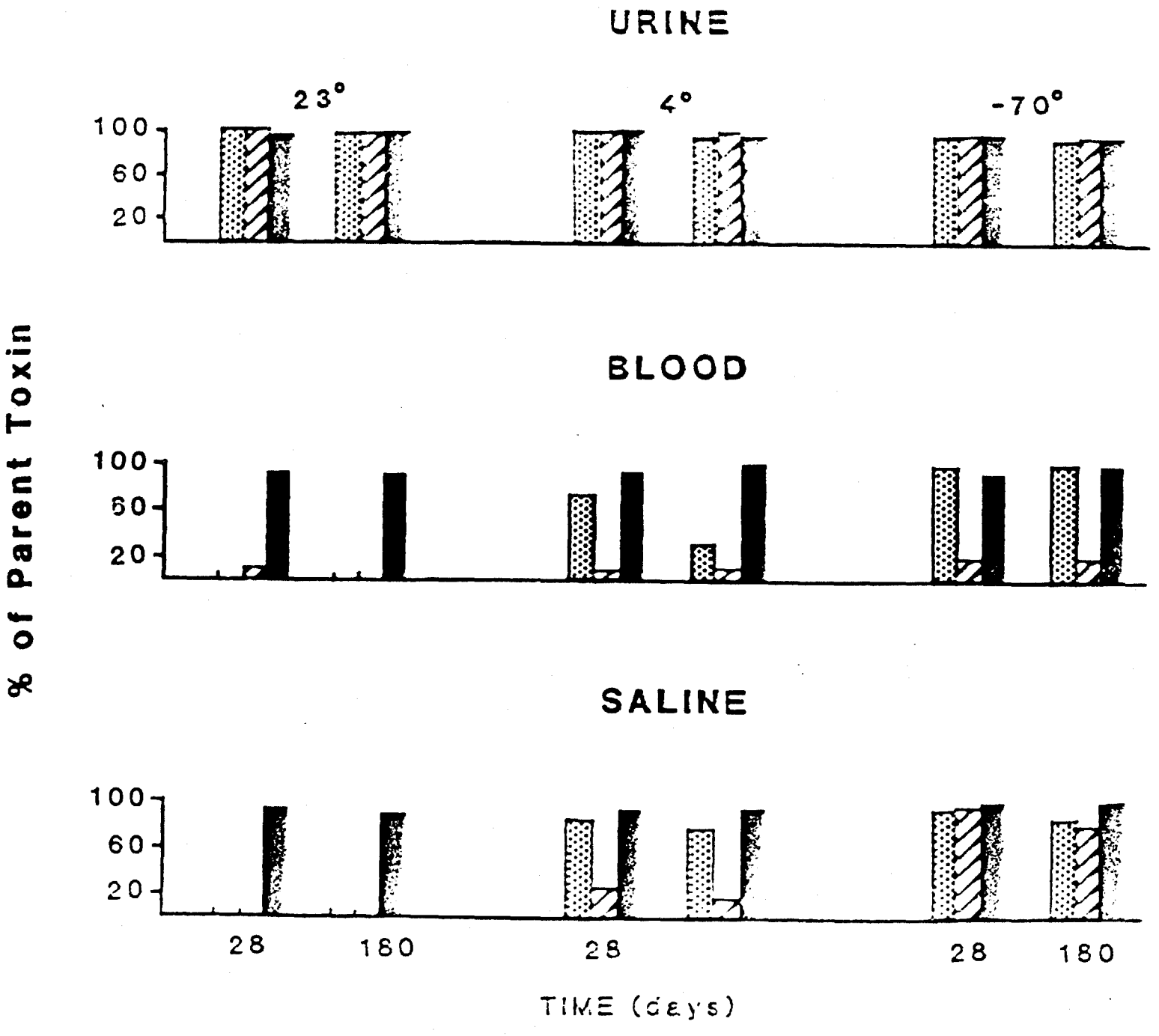
10. **Watson, S. A., C. J. Mirocha, and A. W. Hayes.** 1984. Analysis for trichothecenes in samples from Southeast Asia associated with "yellow rain". Fundam. Appl. Toxicol. 4:700-717.

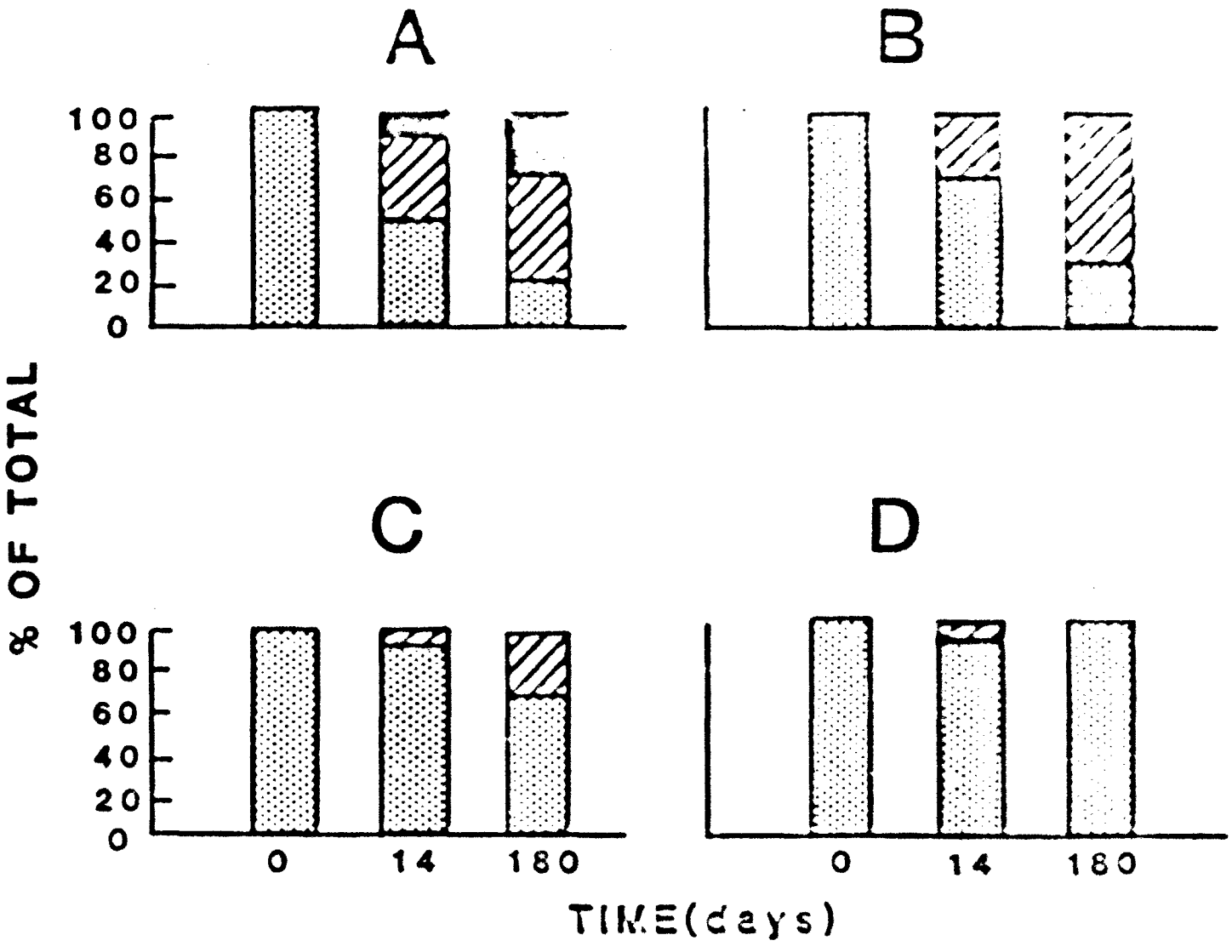
11. **Wei, R.-D., and F. S. Chu.** 1985. Modification of in vitro metabolism of T-2 toxin by esterase inhibitors. Appl. Environ. Microbiol. 50:115-119.

12. **Wei, R.-D., and F. S. Chu.** 1986. Instability of some trichothecenes in methanol. J. Assoc. Off. Anal. Chem. 69:902-903.

13. **Yagen, B., and A. Z. Joffe.** 1976. Screening of toxic isolates of Fusarium poae and Fusarium sporotrichiodes involved in causing alimentary toxic aleukia. Appl. Environ. Microbiol. 32:423-427.

14. **Yoshizawa, T., S. P. Swanson, and C. J. Mirocha.** 1980. T-2 metabolites in the excreta of broiler chickens administered [^{3}H]-labeled T-2 toxin. Appl. Environ. Microbiol. **39**:1172-1177.


URINE
23°
4°
-70°
100
60
20
BLOOD
100
60
20
SALINE
100
60
20
% of Parent Toxin
28
180
28
28
180
TIME (days)


A
B
C
D
100
80
60
40
20
0
% OF TOTAL
0
14
180
TIME(days)